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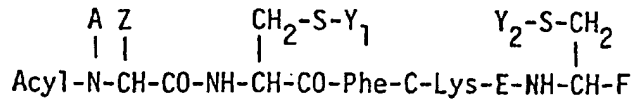
(54) N-Acyl polypeptides

(57) N-Acyl-polypeptides comprising the basic sequence



wherein "Acyl" is the acyl residue of an organic or inorganic acid; A is H or alkyl; >N—CH(Z)—CO— and E are the residues of natural α-amino acids or corresponding (D-amino acids; C is -Trp- or -(D)Trp-; F is a terminal grouping; and Y_1 and Y_2 are each H or together are a direct bond; as well as their salt forms and complexes.

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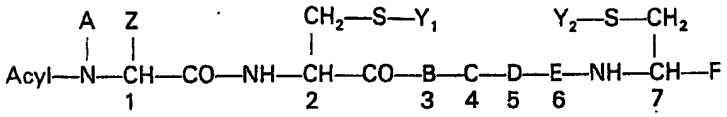
Certain of the Chemical formulae appearing in the printed specification were submitted after the date of filing.

SPECIFICATION

Novel N-acyl-polypeptides and processes for the production thereof

The present invention relates to novel N-acyl-polypeptides, processes for their production, pharmaceutical compositions comprising said N-acyl-polypeptides and their use as pharmaceutically active agents.

More particularly the present invention relates to N-acyl-polypeptides of formula I,



wherein

"Acyl" is the acyl residue of an organic or inorganic acid,

A is hydrogen or C_{1-3} alkyl,

>N—CH(Z)—CO— is

a) an (L)- or (D)-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy, or

b) the residue of a natural α-amino acid other than defined under a) above, or of a corresponding (D)-amino acid,

whereby Z in >N—CH(Z)—CO— represents the remainder of said residue a) or b),

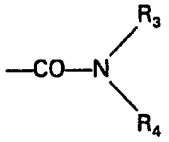
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy,

C is -Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy,

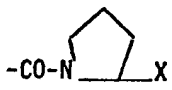
D is -Lys- optionally α-N-methylated and optionally ε-N-C_{1-3} alkylated,

E is the residue of a natural α-amino acid or of a corresponding (D)-amino acid, said residue being optionally α-N-methylated,

F is a group of formula —$COOR_1$, —CH_2OR_2,



or



wherein

R_1 is hydrogen or C_{1-3} alkyl,

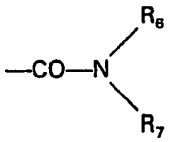
R_2 is hydrogen or the residue of a physiologically acceptable physiologically hydrolysable ester,

R_3 is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl,

R_4 is hydrogen, C_{1-3} alkyl or, when R_3 is hydrogen or methyl, also a group of formula —$CH(R_5)$—X,

R_5 is hydrogen, —$(CH_2)_2$—OH or —$(CH_2)_3$—OH, or represents the substituent attaching to the α-carbon atom of a natural α-amino acid and

X is a group of formula —$COOR_1$, —CH_2OR_2 or



wherein

R_1 and R_2 have the meanings given above,

R_6 is hydrogen or C_{1-3} alkyl and

R_7 is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl,

the group —$CH(R_5)$—X having the (D)- or (L)-configuration, and

Y_1 and Y_2 are each hydrogen or together represent a direct bond, whereby the residues in the 2- and 7-position each independently have the (L) or (D)-configuration, and with the proviso that:

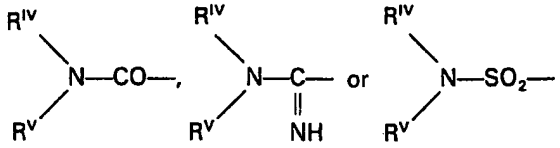
i) (L)- and/or (D)-cysteine residues are present at the 2- and 7-positions only, and

ii) "Acyl" may not represent a residue >N—CH(Z)—CO— as defined above, in which the α-amino group is unsubstituted or is mono- or di-C_{1-12} alkyl substituted,

as well as the salt forms and complexes thereof.

Throughout the present specification and claims by "halogen" is meant fluorine, chlorine and bromine. In accordance with conventional practice, amino acid residues referred to by abbreviation, e.g. -Phe-, -Cys- etc., are to be understood as having the (L)-configuration unless otherwise indicated.

Acyl residues as "Acyl" include, in particular, the acyl residues of organic carboxylic acids, sulfonic acids, sulfaminic acids and carbonic acids and their derivatives. Suitable acyl residues are, e.g. the groups:

1. $R^{I}CO$— wherein R^{I} is an aliphatic, cycloaliphatic, aromatic or heterocyclic group, especially C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl or C_{7-10} (phenylalkyl);
2. $R^{II}SO_2$— wherein R^{II} is C_{1-10} alkyl, phenyl or C_{7-10} (phenylalkyl);
3. $R^{III}O$—CO— wherein R^{III} is C_{1-10} alkyl or C_{7-10} (phenylalkyl); and
4. 

wherein

R^{IV} is hydrogen, C_{1-10} alkyl, phenyl or C_{7-10} (phenylalkyl) and

R^{V} is hydrogen or C_{1-10} alkyl.

Aliphatic groups as R^{I} may be saturated or unsaturated, branched- or straight-chain. Similarly alkyl, alkenyl and alkinyl groups as well as the alkyl-moieties of phenylalkyl groups recited as R^{I} through R^{V} may all be branched- or straight-chain. All groups recited as R^{I} through R^{V} may optionally bear further substituents. Suitably groups recited as R^{I} through R^{V} are unsubstituted.

In the N-acyl-polypeptides of formula I, the following significances or combinations thereof are preferred:

5. "Acyl" is a group $R^{I}CO$— or $R^{II}SO_2$— as defined under 1. and 2. above. Most preferably "Acyl" is a group $R^{I}CO$—.

5.1 When "Acyl" is a group $R^{I}CO$—, R^{I} is preferably C_{1-15} alkyl, phenyl or C_{7-10} (phenylalkyl), more especially C_{1-15} alkyl.

5.2 When "Acyl" is a group $R^{II}SO_2$—, R^{II} is preferably C_{1-10} alkyl or phenyl optionally substituted by C_{1-3} alkyl, especially phenyl or mono- or di-C_{1-3} alkyl-substituted phenyl. Most preferably R^{II} is C_{1-10} alkyl.

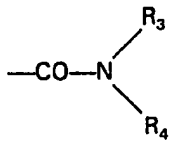
6. A is hydrogen or methyl, especially hydrogen.

7.1 When >N—CH(Z)—CO— has the meaning a), it is preferably an (L)- or (D)-phenylalanine or (L)- or (D)-tyrosine residue (whereby Z is benzyl or p-OH-benzyl), most preferably a (D)-phenylalanine residue.

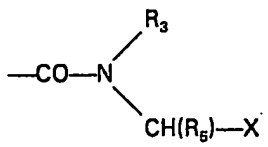
7.2 When >N—CH(Z)—CO— has the meaning b), the defined residue is preferably lipophilic. Preferred residues b) are accordingly residues in which Z is alkyl having 3, preferably 4, or more carbon atoms, in particular the (L)- or (D)-leucine and (L)- or (D)-nor-leucine residues (in which case Z is iso- and n-butyl respectively).

7.3 Most preferably >N—CH(Z)—CO— has the meaning a).

8. B is -Phe-.
9. C is -(D)Trp-.
10. D is -Lys- or -MeLys-, especially -Lys-.
11. E is the residue of a natural α-amino acid, especially -Thr-.
12. F is a group of formula



especially a group of formula

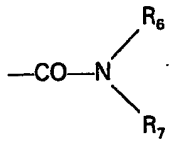


(in which case R_3=H or CH_3). In the latter case the moiety —$CH(R_5)$—X preferably has the L-configuration.

12.1 R_3 is preferably hydrogen.

12.2 As the substituent attaching to the α-carbon atom of a natural amino acid (i.e. of formula H_2N—$CH(R_5)$—COOH), R_5 is preferably —CH_2OH, —$CH(CH_3)$—OH, isobutyl or benzyl, or R_5 is —$(CH_2)_2$—OH or —$(CH_2)_3OH$. It is especially —CH_2OH or —$CH(CH_3)OH$.

12.3 X is preferably a group of formula



—CH_2—OR_2, especially of formula —CH_2OR_2 and R_2 is preferably hydrogen or has the meaning given under 13. below. Most preferably it is hydrogen.

13. As the residue of a physiologically acceptable, physiologically hydrolysable ester R_2 is preferably HCO, C_{2-12} alkylcarbonyl, C_{8-12} phenylalkylcarbonyl or benzoyl.

14. Preferably the residues in the 2- and 7-positions have the (L)-configuration.

15. Preferably Y_1 and Y_2 together represent a direct bond.

A particularly interesting group of N-acyl-polypeptides of formula I are those wherein "Acyl" represents an acyl residue incorporating an aliphatic moiety (e.g. as R^I, R^{II}, R^{III} or R^{IV} of the groups defined under 1. to 4. above) having at least 7, preferably at least 8 carbon atoms, compounds of this type (hereinafter referred to as "N-acyl-polypeptides of Type-T") being characterised by a more prolonged duration of activity when administered sub-cutaneously. Preferred N-acyl-polypeptides of Type-T are those wherein "Acyl" is a group R^ICO— or $R^{II}SO_2$—, especially a group R^ICO—, wherein R^I is C_{7-15} alkyl, preferably C_{7-10} alkyl, especially C_{8-15} alkyl, preferably C_{8-10} alkyl, and R^{II} is C_{7-10} alkyl, especially C_{8-10} alkyl. Especially preferred are N-acyl-polypeptides of Type-T, wherein the remaining residues in formula I have the significances specified under 6. through 15. above.

The N-acyl-polypeptides of the invention may exist in salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. By complexes are to be understood compounds of known type, formed from compounds of formula I on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or on addition of polymeric organic substances.

The present invention also provides a process for the production of the compounds according to the invention. These compounds may be produced by methods known in the art of peptide chemistry or by obvious chemical equivalents thereof, for example by a process comprising:

a) removing the protecting group or groups from a protected N-acyl-polypeptide having the sequence indicated in formula I,

b) linking together by an amide bond two peptide units, each of which contains at least one amino acid or amino alcohol residue in protected or unprotected form, the peptide units being such that a protected or unprotected N-acyl-polypeptide having the sequence indicated in formula I is obtained and, if necessary, carrying out process step a);

c) converting the group F of a protected or unprotected N-acyl-polypeptide having the sequence indicated in formula I, into another group F, and, if necessary carrying out process step a);

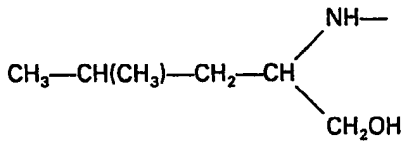
d) oxidising an N-acyl-polypeptide of formula I wherein Y_1 and Y_2 are each hydrogen to provide an N-acyl-polypeptide of formula I, wherein Y_1 and Y_2 together are a direct bond,

and recovering the N-acyl-polypeptide thus obtained in free or salt form or as a complex thereof.

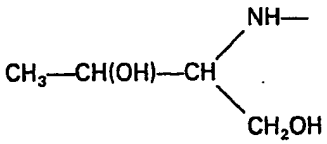
The above process may for example be carried out analogously to the processes described in the accompanying examples. Insofar as the production of the starting materials is not particularly described, the compounds are known or may be produced and purified in accordance with methods known in the art.

In the following examples $[\alpha]_D^{20}$ values are uncorrected. The following abbreviations are employed:

AcOH=acetic acid
AcOEt=ethyl acetate
BOC=tert.-butoxycarbonyl
BTFA=boron-tris-trifluoroacetate
DCCI=dicyclohexylcarbodiimide
DMF=N,N-dimethylformamide
HOBT=N-hydroxybenzotriazole
Leu-ol=the leucinol residue



MBzl=p-methoxybenzyl
Me=methyl
MeOH=methanol
NEt_3=triethylamine
ONP=4-Nitrophenoxy
Phe(pNO_2)=p-NO_2-Phenylalanine
TFA=trifluoroacetic acid
THF=tetrahydrofuran
Thr-ol=the threoninol residue



Z=benzyloxycarbonyl

Example 1

CH_3-(CH_2)$_8$-CO-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

7.1 g CH_3-(CH_2)$_8$-CO-(D)Phe-Cys-(MBzl)-Phe-(D)Trp-Lys(Z)-Thr-Cys-(MBzl)-Thr-ol and 54 ml thioanisole are dissolved in 120 ml TFA at 0° C. The solution is cooled to −10°C, 92 ml ca. 2M BTFA in TFA are added and the solution is stirred for 1.5 hrs. at −10° to −5°C. The obtained reaction mixture is then added with stirring to 400 ml abs. MeOH at −70°C and stirred after 5 minutes with a mixture of 20 ml HCl/ethyl-ether (~5N) in 9 litres absolute ethylether.

The precipitated product is filtered off, washed with ethyl ether and dissolved immediately in 16 litres of dioxane/H_2O (7:3). 4N NH_4OH is added with stirring until the pH reaches 7 to 7.5 and the solution is stirred in an open vessel until testing for —SH groups (e.g. by the Ellmann-method) is negative.

The pH is adjusted to ~3 to 4 by addition of dilute HCl, the solution concentrated under vacuum and lyophylised. The raw product is purified chromatographically on silica gel using a mixture of $CHCl_3$/MeOH/AcOH/H_2O as eluant. Fractions containing the desired product are combined, diluted with H_2O, concentrated and lyophylised to yield the title compound in pure form:

$[\alpha]_D^{20}$=−43.7° (c=0.92 in 95% AcOH).

The starting material is produced as follows:

a) BOC-Cys(MBzl)-Thr-ol

2.1 ml N-methylmorpholine are added with stirring to a solution of 6.3 g Boc-Cys(MBzl)-OH in 50 ml THF, followed by drop-wise addition at −15°C of 2.4 ml chloroformic acid isobutyl ester. After stirring for 5 minutes at −15°C a solution of 3.3 g H-Thr-ol hydrochloride and 4.1 ml N-methylmorpholine in 30 ml DMF, pre-cooled to −10°C is added. The reaction mixture is stirred for 2 hrs. at 0°C and for a further 2 hrs. at room-temperature. 50 ml 10% $KHCO_3$-solution are added and the whole concentrated under vacuum. After dilution with AcOEt, washing 3x with 2N citric acid, 3x with $KHCO_3$-solution and 1x with NaCl solution, the organic phase is dried over Na_2SO_4 and evaporated under vacuum to yield the title compound:

$[\alpha]_D^{20}$=−31° (c=1.3 in DMF).

b) H-Cys(MBzl)-Thr-ol trifluoroacetate

7.1 g BOC-Cys(MBzl)-Thr-ol and 5 ml thioanisole are dissolved in 25 ml methylene chloride. The solution is added to 50 ml TFA and allowed to stand for 20 minutes at room temperature. The mixture is diluted with ca. 1.5 litres ethyl-ether and the precipitated product filtered off and dried to yield the title compound:

$[\alpha]_D^{20}$=8.3 (c=1.1 in 95% AcOH); M.P.=152°C.

c) BOC-Thr-Cys(MBzl)-Thr-ol

5.9 ml chloroformic acid isobutyl-ester are added drop-wise to a solution of 9.7 g Boc-Thr-OH and 9.4 ml N-methyl-morpholine in 50 ml THF pre-cooled to −25°C. The solution is stirred for 5 min. at −15°C and a solution of 20 g H-Cys(MBzl)-Thr-ol trifluoroacetate and 9.8 ml N-methylmorpholine in 50 ml THF, precooled to −10°C are added. The reaction mixture is stirred for 2 hrs. at 0°C, and for 2

hrs. at room temperature. 20 ml 10% $KHCO_3$ are added and the mixture concentrated under vacuum. The product is diluted with AcOEt and washed with 2N citric acid, 10% $KHCO_3$ solution and then 30% NaCl solution. The AcOEt phase is dried over Na_2SO_4 and evaporated under vacuum, and the residue re-crystallised from MeOH/AcOEt/hexane. The title compound is obtained after further filtration, washing with ether and drying:

$[\alpha]_D^{20}$=−23° (c=1 in DMF); M.P.=117°C.

d) H-Thr-Cys(MBzl)-Thr-ol trifluoroacetate

150 ml TFA are added to a solution of 16 g BOC-Thr-Cys(MBzl)-Thr-ol and 17 ml thioanisole in 100 ml methylene chloride precooled to 0°C. The whole is allowed to stand for 20 minutes at room temperature and stirred into ethyl-ether. The precipitated product is filtered off, washed with ethyl-ether and dried to yield the title compound:

$[\alpha]_D^{20}$=+0.6° (c=1 in 95% AcOH); M.P.=72°C.

e) BOC-(D)Trp-Lys(Z)-OMe

9.8 ml NEt_3, 11.8 g HOBT and 21.3 g BOC-(D)Trp-OH are added to 23.3 g H-Lys(Z)-OMe hydrochloride in 300 ml DMF, and the solution cooled to −15°C. 15.6 g DCCI in 50 ml DMF are added and the reaction mixture stirred for ca. 16 hrs. at 0°C, followed by 2 hrs. at room temperature. The reaction mixture is diluted with AcOEt/ethyl-ether and the dicyclohexyl-urea is filtered off. The filtrate is washed with 2N citric acid, H_2O, 10% $KHCO_3$ solution and 30% NaCl solution. The organic phase is dried over Na_2SO_4 and concentrated strongly under vacuum. Crystallisation is effected by the addition of ethyl-ether/hexane and the product is filtered off, washed with ethyl-ether/hexane and dried to yield the title compound:

$[\alpha]_D^{20}$=−12.6° (c=1 in DMF); M.P.=140°C.

f) H-(D)Trp-Lys(Z)-OMe hydrochloride

150 ml TFA are added to 30 g BOC-(D)Trp-Lys(Z)-OMe in 150 ml methylene chloride pre-cooled to 0°C. The whole is stirred for 40 minutes at room-temperature and then added to 30 ml HCl/ethylether (~5N) in 4 l ethyl-ether. After thorough stirring the precipitate is filtered off, washed with ethyl-ether and dried to yield the title compound:

$[\alpha]_D^{20}$=−44° (c=1 in 95% AcOH); M.P.=101°C.

g) BOC-Phe-(D)Trp-Lys(Z)-OMe

9.5 ml NEt_3, 13 g HOBT and 17 g BOC-PHe-OH are added to 35 g H-(D)Trp-Lys(Z)-OMe hydrochloride in 350 ml DMF. The solution is cooled to −20°C and 14.5 g DCCI dissolved in 50 ml DMF are added. The reaction mixture is stirred for ca. 18 hrs. at 0°C and then for 1 day at room temperature. The precipitated dicyclohexyl-urea is filtered off and the filtrate is concentrated, diluted with methanol and H_2O added until precipitation occurs. After filtration the residue is washed with MeOH/H_2O (4:1) and dried to yield the title compound:

$[\alpha]_D^{20}$=+1.1° (c=1 in DMF); M.P=180°C.

h) BOC-Phe-(D)Trp-Lys(Z)-OH

26 ml 1N NaOH are added to a suspension of 16 g BOC-Phe-(D)Trp-Lys(Z)-OMe in dioxane/H_2O (8:2) and the reaction mixture stirred for 1.5 hrs. at room temperature. The obtained solution is diluted to ca. 500 ml by the addition of H_2O and then concentrated under vacuum. The pH is adjusted to 1.5 to 2 by the addition, with stirring, of 1N HCl, the precipitated product is filtered off, washed with H_2O until neutral and dried to yield the title compound:

$[\alpha]_D^{20}$=+7.6° (c=1 in DMF); decomposition at 85—90°C.

i) BOC-Phe-(D)Trp-Lys(Z)-Thr-Cys(MBzl)-Thr-ol

2.5 ml NEt_3, 11.2 g BOC-Phe-(D)Trp-Lys(Z)-OH and 4 g HOBT are added to 9 g H-Thr-Cys(MBzl)-Thr-ol trifluoroacetate in 100 ml DMF. 3.7 g DCCI in 10 ml DMF are added to the solution at −20°C and the whole is stirred for ca. 18 hours at 0°C and 2 hrs. at room temperature. Precipitated dicyclohexyl-urea is filtered off and the filtrate concentrated under vacuum and diluted with methanol. H_2O is added until the product precipitates. The precipitate is filtered, washed with MeOH/H_2O (4:1) and dried to yield the title compound:

$[\alpha]_D^{20}$=−14° (c=1 in DMF); M.P.=135°C.

j) H-Phe-(D)Trp-Lys(Z)-Thr-Cys(MBzl)-Thr-ol hydrochloride

13 g BOC-Phe-(D)Trp-Lys(Z)-Thr-Cys(MBzl)-Thr-ol are dissolved in 60 ml cold TFA/H_2O (15:1) and the solution allowed to stand for 30 min. at room temperature. The reaction mixture is stirred into a mixture comprising 3 litres ethyl-ether and 20 ml HCl in ethyl-ether (~5N) and the precipitated product filtered off, washed with ethyl-ether and dried to yield the title compound:

$[\alpha]_D^{20}$=−3° (c=1 in DMF); decomposition at 110°C.

k) BOC-(D)Phe-Cys(MBzl)-OH

7.73 g BOC-(D)Phe-ONP are added to a solution of 4.83 g H-Cys-(MBzl)-OH in 100 ml dioxane/H_2O (7:3) and 20 ml 1N NaOH and the obtained reaction mixture is stirred for 20 hrs. at room temperature. The reaction mixture is diluted with H_2O and the dioxane removed under vacuum. The aqueous phase is washed with ether and the pH adjusted to 2 by the addition of HCl. The precipitated product is extracted with AcOEt/ethyl-ether, the organic phase washed with water, dried over Na_2SO_4 and evaporated under vacuum to yield the title compound as a foam:

$[\alpha]_D^{20}$=—17° (c=0.9 in DMF).

l) H-(D)Phe-Cys(MBzl)-OH hydrochloride

5 g BOC-(D)Phe-Cys(MBzl)-OH are dissolved in 80 ml TFA and 10 ml H_2O and allowed to stand for 45 minutes at room-temperature. The solution is diluted with ethyl-ether and 20 ml ethyl-ether/HCl (~5N) are added, and the precipitate is filtered off, washed with ethyl ether and dried to yield the title compound:

$[\alpha]_D^{20}$=—38.5° (c=0.93 in 95% AcOH); M.P.=189°C.

m) CH_3-$(CH_2)_8$-CO-(D)Phe-Cys(MBzl)-OH

23.3 ml 1N NaOH are added to 10.0 g H-(D)Phe-Cys(MBzl)-OH hydrochloride in 100 ml dioxane. 7 ml $CH_3(CH_2)_8COCl$ are then added drop-wise with stirring and with simultaneous addition of 1N NaOH, whereby the pH is kept at 8. The obtained reaction mixture is then stirred for a further 20 hrs. at room temperature. The reaction mixture is then adjusted to pH 2 by addition of 4N HCl, concentrated under vacuum, diluted with H_2O and extracted with AcOEt. The organic phase is washed with H_2O, dried over Na_2SO_4 and evaporated under vacuum. The residue is purified chromatographically on silica gel using methylene chloride/MeOH as eluant to yield the title compound:

$[\alpha]_D^{20}$=—19.3° (c=2.6 in DMF). M.P.=124°C.

n) CH_3-$(CH_2)_8$-CO-(D)Phe-Cys(MBzl)-Phe-(D)Trp-Lys(Z)-Thr-Cys(MBzl)-Thr-ol

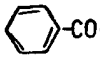
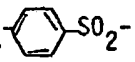
0.7 ml N-methylmorpholine are added to a solution of 5.7 g H-Phe-(D)Trp-Lys(Z)-Thr-Cys(MBzl)-Thr-ol hydrochloride, 0.8 g HOBT and 2.7 g CH_3-$(CH_2)_8$-CO-(D)Phe-Cys(MBzl)-OH in 60 ml DMF. The solution is cooled to —15°C, 1.20 g DCCI in 10 ml DMF are added, and the reaction mixture stirred for 70 hrs. at 0° to 4°C. Precipitated dicyclohexyl-urea is filtered off, the filtrate diluted with MeOH and H_2O added with stirring until the product precipitates. Filtration is effected after ca. 2 hrs., the residue washed with MeOH/H_2O (2:1) and dried under vacuum to yield the title compound:

$[\alpha]_D^{20}$=—20.5° (c=0.8 in DMF).

The following compounds may be produced analogously to the process of example 1 (all compounds listed in the form of the acetate):

Acyl-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

Example No.	"Acyl"	$[\alpha]_D^{20}$ in 95% AcOH
2	CH_3CO-	-42.8° (c = 0.5)
3	$CH_3(CH_2)_4CO$-	-35.8° (c = 0.53)
4	$CH_3(CH_2)_6CO$-	-34.0° (c = 0.40)
5	$CH_3(CH_2)_7CO$-	-35.6° (c = 0.69)
6	$CH_3(CH_2)_{10}CO$-	-36.5° (c = 0.50)
7	$CH_3(CH_2)_{12}CO$-	-27.3° (c = 0.50)

8	t.C_4H_9-CO-	-24.0° (c = 1.0)
9		-33.0° (c = 1.0)
10		-14.3° (c = 1.0)
11	C_2H_5NH-CO-	-32.2° (c = 0.46)
12	D(+)-Biotinyl	-16.9° (c = 1.0)
13	$CH_3(CH_2)_9SO_2$-	-31.0° (c = 0.5)

Acyl-A-Cys-B-(D)Trp-Lys-Thr-Cys-F

Example No.	"Acyl"	A	B	F
14	$CH_3(CH_2)_4CO$-	-(D)Phe(pNO_2)-	-Phe-	-Thr-ol
15	$CH_3(CH_2)_{10}CO$-	-(D)Nle-	-Phe-	-Thr-ol
16	$CH_3(CH_2)_6CO$-	-(D)Phe-	-Phe(pNO_2)-	-Thr-ol
17	$CH_3(CH_2)_{12}CO$-	-(D)Phe-	-Phe-	-(D)Thr-NH_2
18	$CH_3(CH_2)_{12}CO$-	-(D)Phe-	-Phe-	-Phe-OMe
19	$CH_3(CH_2)_{12}CO$-	-(D)Phe-	-Phe-	-Leu-ol

Example 20

Proceeding analogously to examples 1 through 19, but omitting the final oxidisation step, the straight-chain N-acyl-polypeptides corresponding to each of the individual monocyclic polypeptides recited (i.e. wherein the -Cys- residues in the 2- and 7- positions are not linked) are produced. Thus omitting the final oxidisation step from the process of example 1, there is produced the straight-chain N-acyl-polypeptide of formula,

CH_3-$(CH_2)_8$-CO-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol [acetate]
$[\alpha]_D^{20}$ in 95% AcOH=–33.8°; (c=0.42).

N-acyl-polypeptides of the invention as well as their pharmaceutically acceptable salts and complexes exhibit valuable pharmacological properties as indicated in animal tests. In particular they exhibit GH-secretion inhibiting activity as indicated e.g. by depression of serum GH-levels in the rat.

This test is carried out employing male rats under Nembutal narcosis. The N-acyl-polypeptide test-substance is administered s.c. at varying, logarithmically staggered doses in the range of from about 0.1 to 1000 μg/kg, employing at least 4 rats per dose. The rats are decapitated 60 minutes after administration, the blood is collected and the serum GH-level determined by radio-immunoassay.

The above test may be adapted to determine effectiveness over prolonged periods of time, e.g. by decapitating 6 or 18 hours after administration. N-acyl-polypeptides of Type-T as hereinbefore defined are of especial interest, in that they are active in the above described test method when administered at dosages in the aforesaid range, over prolonged periods of time and up to 18 hours.

The said N-acyl-polypeptides, salts and complexes are accordingly indicated for use in the treatment of disorders with an aetiology comprising or associated with excess GH-secretion, e.g. in the treatment of diabetes mellitus and angiopathy as well as of acromegaly.

The said N-acyl-polypeptides, salts and complexes also inhibit gastric- and pancreatic secretion as indicated in standard animal tests, e.g. in accordance with the method described by Doepfner et al., Triangle 16, *2*, 105 (1977) and by Konturek et al., Scand. J. Gastroent. *6*, 423 (1971) also at dosages in the range of from 0.1 to 1000 μm/kg.

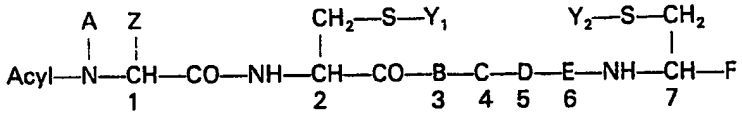
The said N-acyl-polypeptides, salts and complexes are thus further indicated for use in the treatment of gastro-intestinal disorders, for example in the treatment of gastric ulcer, gastro-intestinal bleeding and acute pancreatitis.

For the above uses an indicated daily dose is from about 0.01 mg to about 100 mg N-acyl-polypeptide conveniently administered in divided doses 2 to 4 times a day in unit dosage form or administered in sustained release form. Suitable unit dosage forms contain from about 0.0025 mg to about 50 mg, of an N-acyl-polypeptide in accordance with the invention or equivalent amount of a pharmaceutically acceptable salt or complex thereof, together with a solid or liquid pharmaceutical diluent or carrier therefor.

In accordance with the foregoing the present invention further provides: 1) an N-acyl-polypeptide in accordance with the invention or a pharmaceutically acceptable salt or complex thereof, for use as a pharmaceutical, (e.g. for use as a GH-secretion inhibitor in the treatment of disorders with an aetiology comprising or associated with excess GH-secretion, such as diabetes mellitus, angiopathy and acromegaly or for use as a gastric or pancreatic secretion inhibitor in the treatment of gastro-intestinal disorders such as gastric ulcer, gastro-intestinal bleeding and acute pancreatitis) as well as 2) pharmaceutical compositions comprising an N-acyl-polypeptide in accordance with the invention or a pharmaceutically acceptable salt or complex thereof, together with a pharmaceutically acceptable diluent or carrier therefor.

Claims

1. An N-acyl-polypeptide of formula I,



wherein

"Acyl" is the acyl residue of an organic or inorganic acid,

A is hydrogen or C_{1-3} alkyl,

>N—CH(Z)—CO— is

a) an (L)- or (D) phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy, or

b) the residue of a natural α-amino acid other than defined under a) above, or of a corresponding (D)-amino acid,

wherein Z in >N—CH(Z)—CO— represents the remainder of said residue a) or b),

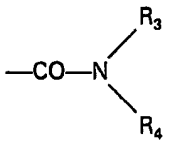
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy,

C is a -Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3} alkyl and/or C_{1-3} alkoxy,

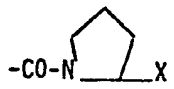
D is -Lys- optionally α-N-methylated and optionally ε-N-C_{1-3} alkylated,

E is the residue of a natural α-amino acid or of a corresponding (D)-amino acid, said residue being optionally α-N-methylated,

F is a group of a formula —$COOR_1$, —CH_2OR_2,



or



wherein

R_1 is hydrogen or C_{1-3} alkyl,

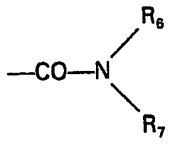
R_2 is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester,

R_3 is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl,

R_4 is hydrogen, C_{1-3} alkyl, or when R_3 is hydrogen or methyl, also a group of formula —$CH(R_5)$—X,

R_5 is hydrogen, —$(CH_2)_2$—OH or —$(CH_2)_3$—OH, or represents the substituent attaching to the α-carbon atom of a natural α-amino acid and

X is a group of formula —$COOR_1$, —CH_2OR_2 or



wherein

R_1 and R_2 have the meanings given above,

R_6 is hydrogen or C_{1-3} alkyl and

R_7 is hydrogen, C_{1-3} alkyl, phenyl or C_{7-10} phenylalkyl,

the group —$CH(R_6)$—X having the (D)- or (L)-configuration, and

Y_1 and Y_2 are each hydrogen or together represent a direct bond, whereby the residues in the 2- and 7-position each independently have the (L) or (D)-configuration, and with the proviso that:

i) (L)- and/or (D)-cysteine residues are present at the 2- and 7-positions only, and

ii) "Acyl" may not represent a residue >N—CH(Z)—CO— as defined above, in which the α-amino group is unsubstituted or is mono- or di-C_{1-12} alkyl substituted,

as well as the salt forms and complexes thereof.

2. An N-acyl-polypeptide according to claim 1, wherein "Acyl" is a group $R^{I}CO$— or $R^{II}SO_2$—, wherein

R^{I} is an aliphatic, cycloaliphatic, aromatic or heterocyclic group, especially C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl or C_{7-10} phenylalkyl, more especially C_{1-15} alkhl, phenyl or C_{7-10} (phenylalkyl); and

R^{II} is C_{1-10} alkyl, phenyl or C_{7-10} (phenylalkyl),

as well as the salt forms and complexes thereof.

3. An N-acyl-polypeptide according to claim 2, wherein R^{I} is C_{7-15} alkyl, especially C_{8-15} alkyl, and R^{II} is C_{7-10} alkyl, especially C_{8-10} alkyl, as well as the salt forms and complexes thereof.

4. An N-acyl-polypeptide according to claim 2 or 3, wherein "Acyl is a group $R^{I}CO$—, as well as the salt forms and complexes thereof.

5. An N-acyl-polypeptide according to claim 1, as well as the acid addition salt forms and complexes thereof.

6. An N-acyl-polypeptide according to claim 1 of formula

$$CH_3\text{-}(CH_2)_8\text{-CO-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol}$$

as well as the salt forms and complexes thereof.

7. Process for the production of a compound according to claim 1, which process comprises

a) removing the protecting group or groups from a protected N-acyl-polypeptide having the sequence defined for formula I in claim I;

b) linking together by an amide bond two peptide units, each of which contains at least one amino acid or amino alcohol residue in protected or unprotected form, the peptide units being such that a protected N-acyl-polypeptide having the sequence defined for formula I in claim 1 is obtained and, if necessary, carrying out process step a):

c) converting the group F of a protected or unprotected N-acyl-polypeptide having the sequence defined for formula I in claim 1, into another group F, and, if necessary carrying out process step a);

d) oxidising an N-acyl-polypeptide of formula I as defined in claim 1, wherein Y_1 and Y_2 are each hydrogen to provide an N-acyl-polypeptide of formula I as defined in claim 1, wherein Y_1 and Y_2 together are a direct bond,

and recovering the N-acyl-polypeptide thus obtained in free or salt form or as a complex thereof.

8. An N-acyl-polypeptide as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or complex thereof for use as a pharmaceutical.

9. An N-acyl-polypeptide or pharmaceutically acceptable salt or complex thereof, according to claim 8 for use in the treatment of disorders with an aetiology comprising or associated with excess GH-secretion or for use as a gastric or pancreatic secretion inhibitor.

10. A pharmaceutical composition comprising an N-acyl-polypeptide as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or complex thereof, together with a pharmaceutically acceptable diluent or carrier therefor.

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